DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

# Memorandum

Date JUN 23 1997

From Deputy Director, Clinical and Review Policy, Office of Device Evaluation (HFZ-400), Center for Devices and Radiological Health

Subject Premarket Approval of Xytronyx, Inc. Periogard Periodontal Tissue Monitor

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Kimber Richter

Kimber C. Richter, M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved X Disapproved _____ Date 6-23-97

Prepared by Carol C. Benson, CDRH, HFZ-440, 6-12-97, 594-1243

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[DOCKET NO. _____]

Xytronyx Inc.; PREMARKET APPROVAL OF Periogard Periodontal Tissue Monitor

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Xytronyx Inc., San Diego, CA, for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of Periogard Periodontal Tissue Monitor. FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter of June 23, 1997, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Alfred Montgomery,

Center for Devices and Radiological Health (HFZ-440),

Food and Drug Administration,

2098 Gaither Rd.,

Rockville, MD 20850,

301-594-1243.

SUPPLEMENTARY INFORMATION: On September 19, 1996, Xytronyx Inc., San Diego, CA 92121, submitted to CDRH an application for premarket approval of Periogard Periodontal Tissue Monitor. The device is a visual, periodontal test kit and is indicated for use as a rapid, chair-side, visual test for the qualitative determination of aspartate aminotransferase (AST) in gingival crevicular fluid. The PTM kit detects elevated levels of AST associated with tissue necrosis. It is intended to be used as an objective, biochemical adjunct to traditional methods of monitoring patients to assist in the decision to apply treatment and in the evaluation of treatment effectiveness.

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Dental Products Panel and/or the Clinical Chemistry and Toxicology Devices Panel of the Medical Devices Advisory Committee, FDA advisory committees, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by the panel. On June 23, 1997, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity for Administrative Review

Section 515(d)(3) of the Federal Food, Drug, and Cosmetic Act (the act), 21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under 21 CFR part 12 of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 21 CFR 10.33(b). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in

brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h) (21 U.S.C. 360e(d), 360j(h))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

JUN 23 1997

Anil K. Singhal Ph.D.
Vice President, Research and Development
Xytronyx Inc.
6730 Mesa Ridge Road, Suite A
San Diego, CA 92121

Re: P960031
Periogard Periodontal Tissue Monitor
Filed: September 19, 1996
Amended: December 3, 1996; February 7, March 10 and 31, and May 6, 1997

Dear Dr. Singhal:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Periogard Periodontal Tissue Monitor (PTM). This device is indicated for use as a rapid, chair-side, visual test for the qualitative determination of aspartate aminotransferase (AST) in gingival crevicular fluid. The PTM kit detects elevated levels of AST associated with tissue necrosis. It is intended to be used as an objective, biochemical adjunct to traditional methods of monitoring patients to assist in the decision to apply treatment and in the evaluation of treatment effectiveness. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Alfred Montgomery, D.V.M., at (301) 594-1243.

Sincerely yours,

Kimber C. Richter

Kimber C. Richter, M.D.
Deputy Director, Clinical and
Review Policy
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

# CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. **This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.**

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mixup of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

(1) may have caused or contributed to a death or serious injury or

(2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Room 240
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

# SUMMARY OF SAFETY AND EFFECTIVENESS DATA

## I. GENERAL INFORMATION

Device Generic Name: Periodontal Test Kit

Device Trade Name: Periogard Periodontal Tissue Monitor

Applicant's Name and Address: Xytronyx Inc.
6730 Mesa Ridge Road, Suite A
San Diego, CA 92121

Date of Panel Recommendation:

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Dental Products Panel and/or the Clinical Chemistry and Toxicology Devices Panel, FDA advisory committees, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

Premarket Approval Application (PMA) Number: P960031

Dates of Good Manufacturing Practice Inspections: 3/11/97, 3/20/97 and 5/8/97

Date of Notice of Approval to Applicant: JUN 23 1997

## II. INDICATIONS FOR USE

The Periogard Periodontal Tissue Monitor (PTM) kit is a rapid, chair-side, visual test for the qualitative determination of aspartate aminotransferase (AST) in gingival crevicular fluid. The PTM kit detects elevated levels of AST associated with tissue necrosis. It is intended to be used as an objective, biochemical adjunct to traditional methods of monitoring patients to assist in the decision to apply treatment and in the evaluation of treatment effectiveness.

### CONTRAINDICATIONS

There are no known contraindications for the Periogard Periodontal Tissue Monitor kit.

WARNINGS AND PRECAUTIONS

Warnings and precautions for use of the device are stated in the attached product labeling. (Attachment 1)

## III. DEVICE DESCRIPTION AND PRINCIPLE OF THE ASSAY

The Periogard Periodontal Tissue Monitor kit (hereinafter called the Periogard) is a visual, periodontal test kit that qualitatively measures the amount of AST in gingival crevicular fluid (GCF). The enzyme AST ( in association with pyridoxal phosphate) in GCF samples is measured visually by a color change reaction. In the reaction, AST catalyzes the transfer of an amino group from cysteinesulfinic acid to alpha-ketoglutarate yielding beta-sulfinyl pyruvate and glutamate. The beta-sulfinyl pyruvate spontaneously and rapidly decomposes and releases inorganic sulfite which reacts with malachite green, simultaneously causing the malachite green to convert from a green colored dye to a colorless form, allowing the pink colored rhodamine B dye to be detected. The rate of conversion of the malachite green is proportional to the AST concentration in the GCF sample. The evaluation of the AST concentration is determined visually. At low concentrations of AST, i.e., $< 1200$ µIU, the test solution remains darker (more violet) than a 1200 µIU reference standard. When elevated concentrations of AST, i.e., $\geq 1200$ µIU, are present, the test solution turns pink (becomes lighter than or equal to the color of the 1200 µIU reference standard).

The following reagents are provided in the kit:

1. Reagent Coated Test Tray - 10 each

   14 well Plastic tray containing dried cysteinesulfinic acid (CSA), malachite green dye, rhodamine B dye, EDTA, polyvinyl alcohol, salts, and various detergents.

2. Crevicular Fluid Sample Paper Units (10 pouches)

   Whatman filter paper placed onto a polyester backing. 16 paper strips per pouch used to collect gingival crevicular fluid (GCF) sample for determination of patient's AST level.

3. Reconstitution Buffer - Bottle A (1 x 16 ml)

   Contains detergents and EDTA in buffer. Used to reconstitute and buffer the reagents in the Reagent Coated Tray.

4. Starter Solution - Bottle B (1 x 6 ml)

Contains α-ketoglutaric acid, pyridoxal phosphate, 0.5% BSA, various detergents and 0.01% sodium azide in buffer. Used to start the AST-catalyzed reaction in the Reagent Coated Test trays

5. AST Standard (+), 1200 μIU - Bottle C (1 x 1 ml)

Contains aspartate aminotransferase (AST), α-ketoglutaric acid, pyridoxal phosphate, 0.5% BSA, various detergents and 0.01% sodium azide in buffer.

6. AST Positive Control (++), 1800 μIU - Bottle D (1x 1 ml)

Contains aspartate aminotransferase (AST), α-ketoglutaric acid, pyridoxal phosphate, 0.5% BSA, various detergents and 0.01% sodium azide in buffer.

## IV. ALTERNATIVE PRACTICES AND PROCEDURES

Alternative practices and procedures for aiding in the monitoring of periodontal diseased patients following treatment include visual examinations, pocket probing examinations, radiographs, and tooth mobility measurements.

## V. MARKETING HISTORY

The Periogard has been marketed in the countries of Canada, The Peoples Republic of China, Italy, the United Kingdom, Greece, Denmark, and France. The Periogard has also been distributed in Western Europe.
The Periogard has not been withdrawn from marketing for any reason relating to the safety and effectiveness of the device.

## VI. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When Periogard is used as an adjunct to conventional clinical methods of monitoring patients who have been previously diagnosed as having periodontal disease and are receiving or have received treatment, there are no known potential adverse effects on the health of patients undergoing management for periodontal disease.

## VIII. SUMMARY OF STUDIES

### NON-CLINICAL STUDIES

Objectives. The objectives of the studies were to test the equivalence, reproducibility, sensitivity, specificity, stability and the stress or wear of the Periogard.

4 - Safety and Effectiveness Data

1. Method Comparison

The accuracy of the Periogard was assessed by comparing the Periogard device to a commercially available method for measuring AST. A series of 5 prepared samples were used for this analysis. Serial dilutions of an AST standard enzyme solution were prepared and the amount of enzyme activity was plotted. The analysis of this method comparison indicated that results obtained with the Periogard method were comparable to those obtained with the commercially available method. Linear regression analysis yielded a correlation coefficient of 0.998, slope of 0.991, y-intercept of 0.477, within the range of 9 to 106 IU/L.

2. Reproducibility Studies

The reproducibility of the Periogard was evaluated at three laboratories, using three testing personnel at each location, using three lots of kits. The study was performed over a three day period at each testing site and using two concentrations of AST that were visually evaluated in comparison with the 1200 $\mu$IU reference standard (+) and the 1800 $\mu$IU positive control (++). The testing personnel were masked from the AST values in the unknown wells containing either 1000 $\mu$IU or 1400 $\mu$IU when compared against the 1200 $\mu$IU reference standard and unknown wells containing either 1500 $\mu$IU or 2100 $\mu$IU AST when compared against a drop of 1800 $\mu$IU positive control. The pooled results from all locations demonstrated > 99 percent correct readings (482/486 and 481/486, respectively) and established that the test method's reproducibility was acceptable. The results are presented in Tables 1 and 2.

*Table 1:* AST Concentrations Compared to the 1200 $\mu$IU Standard (+)

| 1000 $\mu$IU AST Reading | | 1400 $\mu$IU AST Reading | |
|---|---|---|---|
| Positive | Negative | Positive | Negative |
| 0 | 243 | 239 | 4 |

*Table 2:* AST Concentrations Compared to the 1800 $\mu$IU Control (++)

| 1500 $\mu$IU AST Reading | | 2100 $\mu$IU AST Reading | |
|---|---|---|---|
| Positive | Negative | Positive | Negative |
| 0 | 243 | 238 | 5 |

The Periogard uses dropper bottles to dispense the reagents. Studies were conducted to assess the amount of AST delivered to the Reagent Coated Tray from the 1200 μIU Standard (+) and the 1800 μIU Control (++) bottles. Table 3 demonstrates the precision of an AST dispensing bottle.

*Table 3:* Amount of AST delivered to Reagent Coated Tray from 1200 μIU Standard (+) and 1800 μIU Control (++) Bottles.

| Criteria | 1200 μIU Standard (+) | 1800 μIU Control (++) |
|---|---|---|
| N | 432 | 149 |
| Mean | 1265 μIU | 1918 μIU |
| SD | 52 μIU | 68 μIU |
| CV | 4.2 % | 3.5 % |

3. Sensitivity

The minimum assay sensitivity was determined by the lowest concentration of AST activity that could be visually distinguished from a zero AST activity. The lowest detectable concentration was 300 μIU of AST.

4. Specificity

Studies were performed to demonstrate the specificity of the Periogard. Numerous potential interfering substances, which were considered relevant to the sampling of GCF and with a potential impact on the device's functionality were tested. Lysates of a group of normal and pathogenic oral bacteria, possible GCF contaminants (toothpastes, mouthwashes, food particles), GCF enzymes and inflammatory mediators potentially present in periodontitis patients (0.65 nmoles glutathione, 1.65 nmoles cysteine, 2.0 IU lactate dehydrogenase, 10.0 IU amylase, 5.0 IU β-glucuronidase, 0.4 IU collagenase, 56 nmoles prostaglandin E-2), and 20.8 μg tetracycline (a locally-delivered antimicrobial) were shown not to interfere with the test.

Results indicated that serum and saliva were found to contain some AST activity. In addition, hemoglobin was identified as an interfering substance at 10 μG. Contamination by any of these should be avoided during the sampling process so that only clear, GCF samples with no blood or saliva are analyzed.

5. Stability

Stress and Wear Study

Studies demonstrated that multiple exposure to freeze-thaw conditions (10 cycles) caused no significant adverse effects to the performance of the Periogard. The components that were stored under high humidity conditions at 30 C for 5 weeks continued to function. Shipping studies were performed that supported the ruggedness of the Periogard to be transported by overnight courier to destinations, be stored under ambient conditions and returned to the manufacturer without detectable loss of AST activity.

Studies demonstrated that prolonged exposure to light, i.e., 25 hours of simulated solar light and 100 hours of indoor fluorescent light imposed damage to the reagent coated tray, requiring Periogard be stored protected from light.

Real-Time Study

Three lots of Periogard consisting of unique components, i.e., reagent coated trays, reconstituted buffer, starter solution, 1200 $\mu$IU standard, 1800 $\mu$IU control and sample paper were monitored for stability at the recommended storage temperature (2-30 C). Data indicate shelf life stability for 38 weeks.

SUMMARY OF PRECLINICAL STUDIES

Objectives. The objectives of the preclinical studies were to conclude that the measurement of AST, when combined with other standard diagnostic procedures provided an objective measurement permitting improved capacity to distinguish between diseased and non-diseased periodontal sites, and to better assess and monitor the outcome of therapy.

Increases in AST were detected at significant concentrations after ligature-induced periodontitis in beagle dogs, experimental gingivitis in humans, and in patients with periodontitis (1-4). Elevated AST values were found at sites losing 2 mm of clinical attachment (5) and a cross-sectional study found that clinical measures, i.e., bleeding on probing, probing depth and attachment level of past periodontitis and current disease were related to variation in values of CGF AST (6). Sites with less certain disease activity did not demonstrate elevated AST values.

Studies aimed at describing the diagnostic capability of different cutoff values of AST in CGF showed that a 1200 $\mu$IU cutoff gave acceptable positive predictive power (7).

Groups with chronic adult periodontitis and rapid progressive periodontitis had higher

AST values than healthy and marginal gingivitis groups (8). AST values and clinical indicators decreased significantly after non-surgical treatment in systematic healthy and insulin-dependent diabetic patients (9,10).

When the periodontal implant was utilized as the unit of measure, a significant correlation was found between the number of sites with elevated AST values and the number of diseased implants (11).

## CLINICAL STUDIES

### Objectives

The objectives of the clinical studies were to assess the safety and effectiveness of the Periogard in the management and treatment of patients with adult periodontitis.

### Study Design

A multi-center clinical study was conducted at three different clinical centers in the United States, i.e., Harvard University, Boston; University of Washington, Seattle; and University of North Carolina, Chapel Hill, using Periogard to evaluate the association between gingival crevicular AST values and the course of periodontitis progression, or resolution, before and after treatment.

### Subjects Per Investigation

The goal for the study was to enroll 50 periodontal patients and 15 healthy subjects from each of three centers for a total of 150 periodontal patients and 45 healthy subjects. Each healthy subject and periodontitis patient had 8-10 sites monitored.

### Subject Selection and Exclusion

Subjects were to have adult periodontitis as determined by a screening periodontal examination. Periodontitis patients were to present with at least 8 posterior teeth and 8-16 interproximal sites with manual probing depths of $\geq$ 5 mm and $\leq$ 8 mm that had bleeding on probing and bone loss as manifested by $\geq$ 3mm from the cementoenamel junction (CEJ) and alveolar bone crest of bitewing X-rays. Test sites could have been in one or both arches.

Healthy subjects were those without radiographic evidence of periodontal disease with a manual probing depth of $\leq$ 3 mm, a Gingival Index of $\leq$ 1, and no bleeding on probing in all test sites. Age was to be matched as closely as possible with the periodontitis patients.

Subjects were excluded if they had periodontal treatment within the last 6 months; had a history of juvenile periodontitis or acute necrotizing ulcerative gingivitis; were pregnant; had antimicrobial therapy within the last 3 months; had been treated with medications known to affect periodontal status; any disease status which might influence patient compliance; any oral condition likely to require antimicrobial treatment; or presence of untreated caries about the posterior teeth.

Study Population

A total of 166 periodontally-involved subjects with 1381 sites and 47 healthy subjects with 395 sites were enrolled and tested in the 12 month study. At visit 12 there were 127 periodontally involved subjects with 1047 sites tested and 46 healthy subjects with 385 sites tested. Adequate age, gender, and racial distributions in both periodontally-involved and healthy subject groups were achieved. The only demographic category that appeared to influence results was smoking habits. This group had greater disease severity than the group that did not smoke.

Study Period

The study was divided into two phases; a six month pretreatment phase, and a six month posttreatment phase. Periodontally-involved subjects were treated with subgingival scaling and root planing (SRP) at visit six. Healthy subjects received a professional cleaning (prophylaxis) at visit six. GCF sampling and AST testing were performed monthly throughout the study before the monthly examination and treatment at visit six. Standardized radiographs were taken at enrollment, and every three months thereafter. The study was initiated in July 1994 and concluded in March 1996.

Patient Discontinuation

Several subjects missed one or more visits during the study. "Termed" describes those patients terminated from the study for various reasons including missed visits, administration of systemic antimicrobials, or non-compliance with the study protocol. See Tables 4-6

*Table 4:* Harvard University Patient Control:

| | Patients | Controls | Total |
|---|---|---|---|
| Patients Required per Protocol | 50 | 15 | 65 |
| Patients Enrolled in Study | 53 | 15 | 68 |
| Terminated from Study | 11 | 0 | 11 |
| Ending Patients in Study | 42 | 15 | 57 |

9 - Safety and Effectiveness Data

*Table 5:* University of North Carolina Patient Control:

| | Patients | Controls | Total |
|---|---|---|---|
| Patients Required per Protocol | 50 | 15 | 65 |
| Patients Enrolled in Study | 51 | 15 | 66 |
| Terminated from Study | 12 | 0 | 12 |
| Ending Patients in Study | 42 | 15 | 57 |

*Table 6:* University of Washington Patient Control:

| | Patients | Controls | Total |
|---|---|---|---|
| Patients Required per Protocol | 50 | 15 | 65 |
| Patients Enrolled in Study | 62 | 17 | 79 |
| Terminated from Study | 23 | 1 | 24 |
| Ending Patients in Study | 43 | 16 | 59 |

Individual sites were terminated from the study if they experienced $\geq 2$ mm of attachment loss.

Adverse Reactions and Complications

During the course of the clinical study the applicant did not become aware of any adverse reactions or complications associated with the use of the Periogard.

Patient Assessments

Traditional clinical parameters, i.e., bleeding on probing (BOP), gingival index (GI) and pocket depth (PD) in addition to GCF AST were measured monthly in periodontally involved subjects and every three months in healthy subjects to access the response of each parameter before and after treatment at six months. The Florida Probe was used to measure relative attachment levels and the cephalographic x-ray method for digital subtraction radiography (DSR) was used to assess bone density.

Data Analysis and Results

AST as well as other clinical measurements (mean pocket depth, bleeding on probing, gingival index, bone height loss and bone density loss) exhibited change over time. The trend was for periodontally-involved subjects to improve in these measures as they moved from month 0 to month 6 to month 12.

At the initial visit, there was a significant difference ($p < 0.001$) between the percent of AST positive sites for the periodontally-involved group and the healthy group (50% and 14%, respectively). Periodontally-involved subjects had a significantly greater percent of AST positive sites throughout the study. At a patient level, it was found that the frequency of AST positive sites at the initial visit was higher in the periodontally-involved subjects (see Table 7). For example, 72.9% of periodontally-involved subjects had ≥3 AST positive sites, while 19.2% of healthy subjects had ≥3 sites that were AST positive.

*Table 7:* Frequencies of Study Subjects with Greater than 1,2,3 and 4 AST Positive Sites at Initial Visit out of 8-10 Sites Sampled

| | Percent of Subjects at Initial Visit with AST Positive Sites | | | |
|---|---|---|---|---|
| Subject Group | ≥ 1 | ≥ 2 | ≥ 3 | ≥ 4 |
| Healthy (N=47) | 48.9 % | 31.9 % | 19.2 % | 10.6 % |
| Periodontally-Involved (N=166) | 93.4 % | 81.3 % | 72.9 % | 59.0 % |

The percent of AST positive sites decreased after treatment in both periodontally-involved and healthy subjects (see Table 8). Within one month of the treatment (Tx), a reduction in the percent AST positive sites was observed in the periodontally-involved subjects and continued to decrease until the end of the study.

*Table 8:* Percent AST Positive Changes in Response to Therapy (Periodontally-InvolvedandHealthy Subjects)

| Time Post - Tx (months) | % AST Positive in Periodontally-Involved Subjects | % AST Positive in Healthy Subjects |
|---|---|---|
| 0 | 40.8 % | 20.7% |
| + 1 | 30.9 % | N/A |
| + 3 | 25.2 % | 11.7% |
| + 6 | 22.8 % | 8.0% |

Device Failures and Replacements

During the course of the clinical study the applicant did not provide any product replacements as a result of the Periogard not performing to product specifications.

## VIII. CONCLUSIONS DRAWN FROM THE STUDIES

### RISK BENEFIT ANALYSIS

The Periogard presented no significant risk to the patient. With the exception of the minimally invasive GCF sampling procedure, all steps and procedures were performed external to the patient.

The benefit of the Periogard is the identification of sites in need of treatment or sites that will not be as responsive to therapy. Early identification may result in less aggressive treatment being used than would be required if clinically measurable disease progression occurs and more rounds of treatment or more aggressive treatment are required to avert further disease progression.

## IX. PANEL RECOMMENDATION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Dental Products Panel and/or the Clinical Chemistry and Toxicology Devices Panel, FDA advisory committees, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

## X. CDRH DECISION

On March 4, 1997, FDA requested clarification and additional information as follows: 1) a table summarizing the patients that did not complete the study, 2) clarification of the discrepancies in patient accountability and 3) revisions to the labeling.

Xytronyx Inc. submitted the information. FDA issued an approval order on .
The sponsor's manufacturing facilities were inspected on 3/11/97, 3/20/97, and 5/8/97 and were found to be in compliance with the device Good Manufacturing Practice regulations.

## XI. APPROVAL SPECIFICATIONS

Directions for use: See the labeling (Attachment 1).

Hazards to Health from Use of the Device: See Indications, Warnings, Precautions and Adverse Events in the labeling (Attachment 1).

References:

1. Chambers DA, Crawford JM, Mukherjee S, and Cohen RL: Aspartate aminotransferase increases in crevicular fluid during experimental periodontitis in beagle dogs. J Periodontol 55:526-530, 1984.

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ATTACHMENT 1:

## PERIODONTAL TISSUE MONITOR PACKAGE INSERT
## Kit Size: - 10 Patients

### 5.2.1 INTENDED USE

A rapid, chair-side, visual test for the qualitative determination of aspartate aminotransferase (AST) in gingival crevicular fluid.

### 5.2.2 INDICATIONS FOR USE

The Periodontal Tissue Monitor (PTM) kit is a rapid, chair-side, visual test for the qualitative determination of aspartate aminotransferase (AST) in gingival crevicular fluid. The PTM kit detects elevated levels of AST associated with tissue necrosis. It is intended to be used as an objective, biochemical diagnostic adjunct to traditional methods of monitoring patients to assist in the decision to apply treatment and in the evaluation of treatment effectiveness.

**Warnings:**

- Federal Law restricts this device to sale, distribution or use by or on the order of a licensed dentist or physician.
- AST Standard, AST Positive Control and Starter Solution contain sodium azide. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Large quantities of water must be used to flush solutions down a sink. See reference #13 for appropriate handling.

**Precautions:**

- For *In Vitro* diagnostic use.
- Test results must be read by a dental professional.
- DO NOT use the materials after the expiration date.
- DO NOT interchange kit components with other kits that have different lot numbers.
- Prolonged exposure of the components to light (>100 hours under fluorescent lighting) has been found to have a deleterious effect on the functional performance of the test. Kit components should be stored in the box protected from light.

## 5.2.3 SUMMARY AND EXPLANATION

Traditional techniques used in the diagnosis of periodontal disease and in monitoring the outcome of therapy include pocket depth, attachment level, gingival redness, bleeding on probing, and assessment of alveolar bone on radiographs. Clinical studies performed in the 1980's demonstrated the possibility that disease-active and disease-inactive periodontal pockets exist (10). However, traditional diagnostic procedures cannot distinguish between disease-active and inactive states. Additional diagnostic information is needed to assist in the decision of when to apply treatment and whether the treatment rendered was effective. The Periodontal Tissue Monitor (PTM) kit, detects elevated levels ($^3$1200 µIU) of aspartate aminotransferase (AST) in gingival crevicular fluid (GCF) and is intended to be used as an objective, biochemical adjunct to traditional methods of diagnosing and monitoring patients in determining when to treat and evaluating treatment effectiveness.

AST is an enzyme that is found in all cells, although its levels vary between different cell types. Cells release AST into the extracellular space during periods of tissue necrosis or tissue trauma, resulting in detectable quantities of the enzyme being carried into peripheral circulation. In this way, elevated serum levels of AST have been detected and used in the diagnosis and treatment of certain types of liver and heart diseases. (1, 2). Indeed, AST is found in GCF at elevated levels in diseased sites compared to healthy sites (7, 8). It has also been demonstrated that support tissues in the periodontium, i.e., the gingival epithelial cells, gingival fibroblasts and periodontal ligament fibroblast cells contain significant levels of AST (100, 60 and 20 KU/1000 cells respectively) (6).

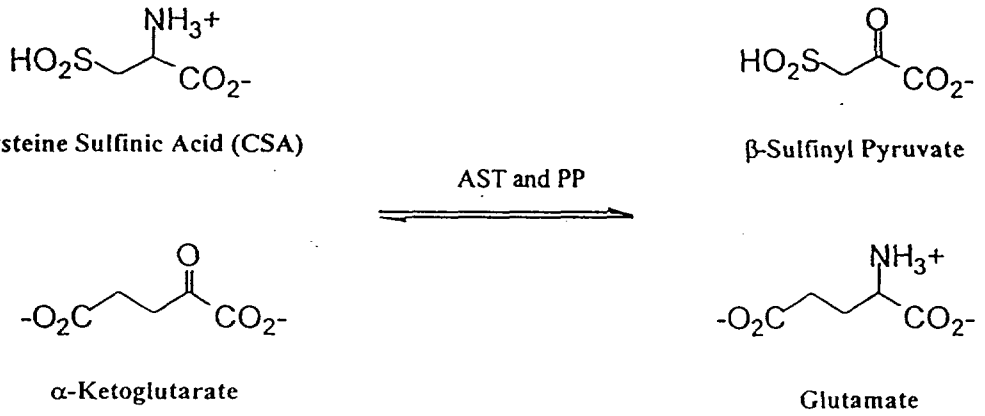
Human studies aimed at describing the diagnostic capability of different cutoff levels of AST in GCF concluded that a 1200 µIU cutoff gave positive predictive power (5). Other clinical studies concluded that elevated levels of AST are associated with acute gingival inflammation (7), increased GCF volume (7) and attachment loss (4, 5). AST levels also dropped significantly in response to traditional therapies such as scaling and root planing (7, 8). Moreover, many studies have concluded that elevated levels of AST in GCF are suggestive of periodontal disease activity (3, 4, 7).

Historically, AST is a reliable measure of tissue necrosis and AST levels in GCF have been shown to increase during episodes of periodontal tissue destruction in clinical studies involving healthy and periodontally-involved patients. The level of AST in GCF has also been shown to decrease in response to therapy. These results suggest that elevated AST is associated

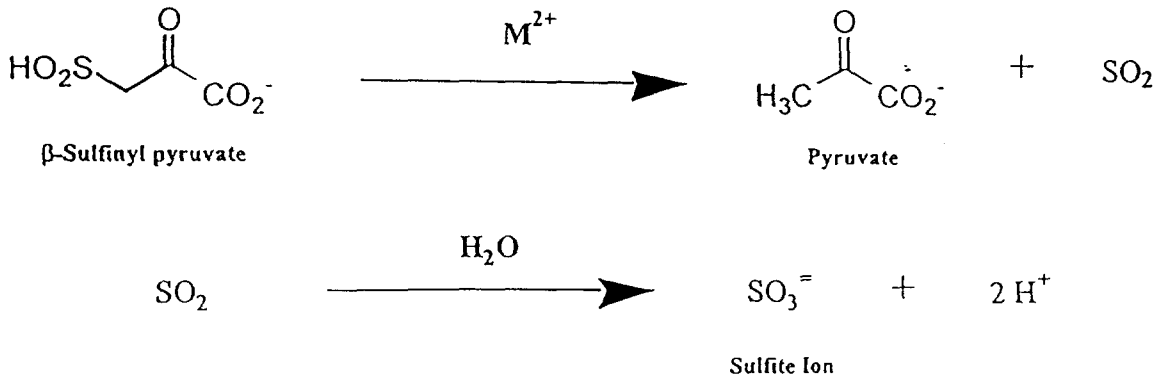
with tissue necrosis present in disease and provides adjunctive clinical information in assessing treatment efficacy. Two tests at an appropriate interval are necessary to make this assessment.

## 5.2.4 PRINCIPLES

Cysteinesulfinic acid (CSA) is accepted as a structural analog of aspartic acid (aspartate) by aspartate aminotransferase (AST). The scientific literature has reported that CSA is an alternative substrate for AST. Similar to the Bergmeyer method, AST [in association with PP (pyridoxal phosphate)] catalyzes the transfer of an amino group from CSA to alpha-ketoglutarate to yield beta-sulfinyl pyruvate and glutamate.

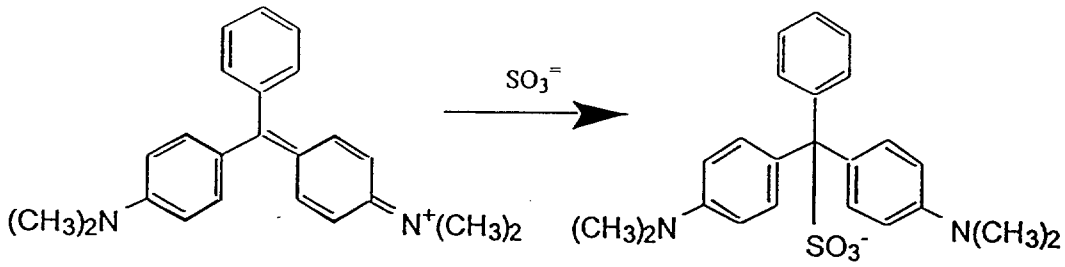



AST catalyzes the formation of beta sulfinyl pyruvate as described above. Beta sulfinyl pyruvate spontaneously and rapidly decomposes and releases inorganic sulfite.




Sulfite ion reacts with malachite green (MGC), simultaneously causing MGC to convert from a green colored dye to its leuco or colorless form,

allowing the pink colored rhodamine B dye to show through. The rate of conversion of the MGC is directly proportional to AST concentration.




**Malachite Green**

**Malachite Green Sulfite Adduct (Colorless)**

The evaluation of the AST concentration is determined visually. At low concentrations of AST, i.e., < 1200 µIU, the test solution remains darker (more violet) than a 1200 µIU reference standard. When elevated concentrations of AST, i.e., ≥ 1200 µIU, are present, the test solution turns pink (becomes lighter than or equal to the color of the 1200 µIU reference standard).

## 5.2.5 GENERAL INFORMATION

There is sufficient material supplied with each Periodontal Tissue Monitor kit (PN 100-0008) for 10 patient tests; up to 12 gingival sites may be evaluated per test.

**Reagents and Materials Provided**:

1. <u>Reagent Coated Test Tray - 10 each</u>

14 well Plastic tray containing dried cysteinesulfinic acid (CSA), malachite green dye, rhodamine B dye, EDTA, polyvinyl alcohol, salts, and various detergents.

2. <u>Crevicular Fluid Sample Paper Units (10 pouches)</u>

Whatman filter paper placed onto a polyester backing. 16 paper strips per pouch used to collect gingival crevicular fluid (GCF) sample for determination of patient's AST level.

3. Reconstitution Buffer - Bottle A (1 x 16 ml)

Contains detergents and EDTA in buffer. Used to reconstitute and buffer the reagents in the Reagent Coated Tray.

4. Starter Solution - Bottle B (1 x 6 ml)

Contains α-ketoglutaric acid, pyridoxal phosphate, 0.5% BSA, various detergents and 0.01% sodium azide in buffer. Used to start the AST-catalyzed reaction in the Reagent Coated Test trays.

5. AST Standard (+), 1200 µIU - Bottle C (1 x 1 ml)

Contains aspartate aminotransferase (AST), α-ketoglutaric acid, pyridoxal phosphate, 0.5% BSA, various detergents and 0.01% sodium azide in buffer.

6. AST Positive Control (++), 1800 µIU - Bottle D (1x 1 ml)

Contains aspartate aminotransferase (AST), α-ketoglutaric acid, pyridoxal phosphate, 0.5% BSA, various detergents and 0.01% sodium azide in buffer.

7. Package Insert (1 each)

**Materials needed but not provided with kit:**

- Light Box - used to assist with reading reagent coated tray
- Timer with minute readout - used to time reaction step
- Forceps - used to place Crevicular Fluid Sample Paper strips

**Storage:**

- All components provided with the Periodontal Tissue Monitor kit can be stored at room or refrigerated temperatures (2°-30°C; 36°-86°F).
- Components must be stored protected from light.
- Reagent Coated Trays can not be stored once the seal is broken.
- Crevicular Fluid Sample Paper can not be stored once the seal is broken.
- Bottled reagents can, however, be stored once used.

**Procedural Limitations:**

- Test only clear GCF samples containing NO BLOOD or SALIVA. The presence of blood or saliva may interfere with the test.
- Sample before ANY manipulation of the sulcus, such as probing, prophylaxis, root planing, etc. Mechanical stimulation of gingival tissue may alter the test outcome.
- Sample after waiting at least one hour if tooth brushing occurred.
- Sample sites no more than once per day.
- Sample within 15 minutes of adding reconstitution buffer to the test well.

5.2.6 **TEST PROCEDURE**

**A. Selection of Sites**

**Step 1** Sites are selected based upon clinical manifestations of the disease, such as pocket depth, severe gingival inflammation, attachment loss or radiographic evidence of bone loss (9).

**Step 2** When sites cannot be selected on this basis, selection should favor the six Ramfjord sites (11) or the teeth at greatest risk or sites that have been treated for previous disease.

**Step 3** Label the wells of the PTM kit tray with a permanent marker to correspond to the selected test sites.

**B. Reagent Coated Tray Preparation**

**Step 1** To ensure accurate delivery volumes, hold each bottle in the vertical position when dispensing fluids. Prepare the test tray by adding 3 drops of Reconstitution Buffer (**Bottle A**) to each test well.

**Step 2** Dissolve the dye in each of the wells by gently agitating the test tray (be careful not to spill any fluid) for 20 seconds or until there is complete dissolution. A light box may be used to check for complete dissolution.

***Precaution: Patient samples must be collected within 15 minutes of adding the Reconstitution Buffer (Bottle A).***

**C. Sample Collection**

**Step 1** Thoroughly dry the area around the test site by using

a gentle application of forced air then isolate the test site with cotton. This will prevent dilution of the test sample by saliva, which could result in a false negative reading.

**Step 2** Remove one unit of 16 crevicular fluid sample paper strips from its plastic pouch. Peel off the adhesive covers to attach the crevicular fluid sample paper holder to a convenient location in the work area or to place around the finger. Each crevicular fluid sample paper strip has a red plastic coated end for holding and an absorbent end for crevicular fluid sample collection.

**Step 3** Using forceps, grasp one of the crevicular fluid sample paper strips by the red plastic coated end and gently place the absorbent end at the orifice of the gingival sulcus to collect the gingival crevicular fluid (GCF) sample. **The sample paper strips should not be forced into the sulcus pocket.** A slight jiggling motion may facilitate placement.

**Step 4** Let the GCF absorb into the crevicular fluid sample paper strip for **30 seconds**. Place the crevicular fluid sample paper strip containing the GCF sample in its corresponding sample well. This procedure is repeated for each site sampled (multiple sites may be sampled simultaneously). Discard unused strips.

***Precaution: Samples visibly contaminated with blood or saliva must be discarded.***

## D. Performing the Test

**Step 1** After all of the test sites have been sampled and their respective crevicular fluid sample paper strips are in the appropriate sample wells, start the reaction by adding one drop of Starter Solution **(Bottle B)** to each test well **except the control wells (+), (++).**

***Precaution: No Starter Solution(Bottle B) should be added to the reference standard ("+") or positive Control ("++") wells.***

**Step 2** Immediately after adding the Starter Solution **(Bottle B)** to the sample wells, add one drop of the 1200 µIU AST Standard Solution **(Bottle C)** to the well marked "+" and add one drop of the 1800 µIU AST Positive Control **(Bottle D)** to the "++" well. Mix by gently agitating the test tray for 10 seconds. Agitate the tray two to

three times during the course of the reaction.

## E. Reading and Scoring Test Result

**Step 1** Score the test when the AST Standard "+" well begins to turn pink *:

**Positive Result:**
A sample is *positive* if at any time after addition of the Starter Solution it is equal to or lighter (more pink) than the AST Standard "+" well.

**Negative Result:**
A sample is considered *negative* if it is darker (more violet) than the AST standard "+" well.

**Invalid Result:**
A sample is considered *invalid* if the AST positive control "++" well does not change color before the AST standard "+" well within the appropriate time frame (see Table 1). An invalid result indicates that either the test was not performed correctly or that the reagents are not working properly.

* Notes: At temperatures of 22-25°C (70-77°F), the AST Positive Control **(Bottle D)** should change color within about **4 minutes** and the AST Standard **(Bottle C)** should be ready to compare to test samples within about **6 minutes**. (However, test samples should be scored after no longer than 10 minutes. After a period of time all wells will turn pink, making interpretation impossible.)

As the room temperature increases the rate of the enzymatic reaction increases and, hence, the color changes sooner. The converse occurs as the room temperature decreases.

*Table 1: Reading Time Guideline for samples compared to 1200 µIU Standard (+) and 1800 µIU Control (++)*

| | Read Results Before: | |
|---|---|---|
| **Temperatures** | **1800 µIU AST Control (++)** | **1200 µIU AST Standard (+)** |
| 16°C (61°F) | 12 min | 12 min |
| 23°C (73°F) | 7 min | 10 min |
| 30°C (86°F) | 5 min | 6 min |

## 5.2.7 INTERPRETATION OF TEST RESULTS

AST results with the PTM kit may be interpreted as described below to provide additional objective, biochemical information used to diagnose and monitor patients during the course of periodontal treatment (12).

**AST Positive Result*** This is a site with an elevated level of tissue necrosis. A positive result and other clinical signs of disease supports a decision to institute appropriate treatment or re-treatment.

**AST Negative Result** * This is a site without elevated tissue necrosis and in the absence of clinical signs of disease, indicates periodontal health.

* As is the case with any diagnostic procedure, the results obtained by this kit yield data that must be used only as an adjunct to other clinical information available to the dental professional.

## 5.2.8 EXPECTED VALUES

The results of a multi-center clinical study completed in 1996 serve to illustrate typical results which can be expected from the Periodontal Tissue Monitor kit (12). In this study over a 12 month period, 1,381 sites in 166 periodontally -involved subjects and 395 sites in 47 healthy subjects were enrolled and tested. Monthly testing was performed on the periodontally-involved subjects with 1,047 sites (127 subjects) completing the study. Healthy subjects were tested every three months with 385 sites (46 subjects) completing the study. Treatment was rendered at the 6 month time point and involved scaling and root planing (SRP) for the periodontally-involved subjects and a prophylaxis for healthy subjects. AST results were obtained at each visit throughout the study in addition to other clinical parameters, i.e., pocket depth (PD), bleeding on probing (BOP), gingival index (GI) and attachment level (AL). Digital subtraction radiography was performed every three months on all subjects.

At the initial visit, there was a significant difference ($p < 0.001$) between the percent of AST positive sites for the periodontally-involved group and the healthy group

(50% and 14%, respectively). Periodontally-involved subjects had a significantly greater percent of AST positive sites throughout the study.

At a patient level, it was found that the frequency of AST positive sites at the initial visit was higher in the periodontally-involved subjects (see Table 2). For example, 72.9% of periodontally-involved subjects had ≥3 AST positive sites, while 19.2% of healthy subjects had ≥3 sites that were AST positive.

*Table 2:* Frequencies of Study Subjects with Greater than 1,2,3 and 4 AST Positive Sites at Initial Visit out of 8-10 Sites Sampled

| | **Percent of Subjects at Initial Visit with AST Positive Sites** | | | |
|---|---|---|---|---|
| **Subject Group** | **≥ 1** | **≥ 2** | **≥ 3** | **≥ 4** |
| Healthy (N=47) | 48.9 % | 31.9 % | 19.2 % | 10.6 % |
| Periodontally-Involved (N=166) | 93.4 % | 81.3 % | 72.9 % | 59.0 % |

The percent of AST positive sites decreased after treatment in both periodontally-involved and healthy subjects (see Table 3). Within one month of the treatment (Tx), a reduction in the percent AST positive sites was observed in the periodontally-involved subjects and continued to decrease until the end of the study.

*Table 3:* Percent AST Positive Changes in Response to Therapy (Periodontally-Involved and Healthy Subjects)

| **Time Post - Tx (months)** | **% AST Positive in Periodontally-Involved Subjects** | **% AST Positive in Healthy Subjects** |
|---|---|---|
| 0 | 40.8 % | 20.7% |
| + 1 | 30.9 % | N/A |
| + 3 | 25.2 % | 11.7% |
| + 6 | 22.8 % | 8.0% |

## 5.2.9 PERFORMANCE CHARACTERISTICS

Readability and Reproducibility

Assay readability and reproducibility were evaluated using three lots of PTM components at three locations, with three different readers, during three different testing periods (see Tables 4 & 5). 1000 µIU and 1400 µIU levels of AST were read against the 1200 µIU Standard (+) and 1500 µIU and 2100 µIU levels of AST were read against the 1800 µIU Control (++). The pooled results from all locations demonstrated > 99.0% correct readings (482/486 and 481/486 respectively).

*Table 4:* AST Concentrations Compared to the 1200 µIU Standard (+)

| **1000 µIU AST Reading** | | **1400 µIU AST Reading** | |
|---|---|---|---|
| Positive | Negative | Positive | Negative |
| 0 | 243 | 239 | 4 |

*Table 5:* AST Concentrations Compared to the 1800 µIU Control (++)

| **1500 µIU AST Reading** | | **2100 µIU AST Reading** | |
|---|---|---|---|
| Positive | Negative | Positive | Negative |
| 0 | 243 | 238 | 5 |

In the context of the PTM test, the rate of AST substrate conversion is dependent on the AST concentration and temperature at which the test is performed. A study was conducted to determine a general guideline for the time vs. temperature window of readability for the PTM kit (See Table 1).

Interference Testing

Lysates of a group of normal and pathogenic oral bacteria, possible GCF contaminants (toothpastes, mouthwashes, food particles), GCF enzymes and inflammatory mediators potentially present in periodontitis patients (0.65 nmoles glutathione, 1.65 nmoles cysteine, 2.0 IU lactate dehydrogenase, 10.0 IU amylase, 5.0 IU β-glucuronidase, 0.4 IU collagenase, 56 nmoles prostaglandin E-2), and 20.8

µg tetracycline (a locally-delivered antibiotic) were shown not to interfere with the test.

Precision

The PTM kit uses dropper bottles to dispense the reagents. Studies were conducted to assess the amount of AST delivered to the Reagent Coated Tray from the 1200 µIU Standard (+) and the 1800 µIU Control (++) bottles. Table 6 demonstrates the precision of an AST dispensing bottle.

*Table 6:* Amount of AST delivered to Reagent Coated Tray from 1200 µIU Standard (+) and 1800 µIU Control (++) Bottles.

| Criteria | 1200 µIU Standard (+) | 1800 µIU Control (++) |
|---|---|---|
| N | 432 | 149 |
| Mean | 1265 µIU | 1918 µIU |
| SD | 52 µIU | 68 µIU |
| CV | 4.2 % | 3.5 % |

## 5.2.10 REFERENCES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5. Persson GR, Page RC: Diagnostic characteristics of crevicular fluid aspartate aminotransferase (AST) levels associated with periodontal disease activity. J Clin Periodontol 19:43-48, 1992.

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12. Data on file: Xytronyx, Inc. San Diego, CA. 92121

13. Decontamination of Sink Drains to Remove Azide Salts. Safety Management No. CDC-22. Centers for Disease Control, Atlanta, Georgia. April 30, 1976.

### 5.2.11 **Name and Place of Business of Manufacturer**

***Xytronyx, Inc.***
6730 Mesa Ridge Rd. Suite A
San Diego, CA 92121 USA
Tel: 619-550-3900
FAX: 619-550-3929
U.S. Patents 5,047,328 & 5,041,373
Other U.S. & International Patents Pending
910-0015.A; Issue Date: ###